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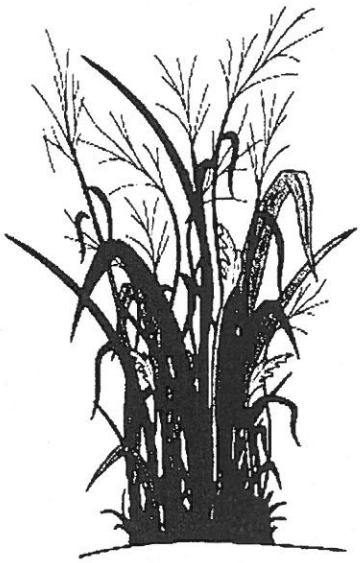

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Characterization of the GAUT1- and GAUT7-containing Arabidopsis Pectin Biosynthetic Enzyme Complex

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GAlactUronosylTransferase 1 (GAUT1) is an Arabidopsis pectin biosynthetic alpha-1,4-galacturonosyltransferase (GalAT) that transfers galacturonic acid (GalA) from uridine diphosphate-GalA (UDP-GalA) onto exogenous acceptors of homogalacturonan (HG) (1). Multiple lines of evidence, including co-immunoprecipitation using rabbit polyclonal anti-GAUT1 or anti-GAUT7 antibodies and Bimolecular Fluorescent Complementation experiments, show that GAUT1 exists in a protein complex with GAUT7, another member of the GAUT gene family. To further characterize this pectin biosynthetic enzyme complex, we used GAUT1- and GAUT7-specific IgG conjugated to magnetic beads to affinity-purify the protein complex. The immunoabsorbed proteins were eluted from the beads, separated on SDS-PAGE, and analyzed by in-gel trypsin digestion and subsequent liquid chromatography – tandem mass spectrometry (LC-MS/MS) to identify the components of the complex. The results verified the presence of GAUT1 and GAUT7 in the immunoabsorbed protein mixtures, and identified several other proteins, including putative methyltransferases, a couple of members of the oligosaccharyltransferase complex, and an unknown protein similar to N-acetyltransferase from other organisms, as candidate members of the GAUT1:GAUT7 enzyme complex. This evidence of a novel, multi-subunit wall biosynthetic enzyme complex may shed light to elucidating the mechanism of pectin synthesis. A model for the GAUT1:GAUT7 core complex is also presented. This research is funded by NRI, CSREES, USDA Awards 2003-35318-15377 and 2006-35318-17301.

(1) Sterling, J.D., Atmodjo, M.A., Inwood, S.E., Kumar Kolli V.S., Quigley, H.F., Hahn, M.G., and Mohnen, D. (2006) PNAS 103(13):5236-41.